

## Research report

# The CRF<sub>1</sub> receptor antagonist SSR125543 prevents stress-induced long-lasting sleep disturbances in a mouse model of PTSD: Comparison with paroxetine and D-cycloserine

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## HIGHLIGHTS

- The CRF<sub>1</sub> antagonist SSR125543 attenuates the long-term effects of stress.
- Sleep disturbances are commonly reported symptoms in post-traumatic stress disorder.
- Here we tested SSR125543 on sleep impairment induced by traumatic stress using EEG.
- The stress-induced effects were prevented by repeated administration of SSR125543.
- These findings confirm that SSR125543 can attenuate the effects of traumatic stress.

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## ABSTRACT

The selective CRF<sub>1</sub> (corticotropin releasing factor type 1) receptor antagonist SSR125543 has been previously shown to attenuate the long-term behavioral and electrophysiological effects produced by traumatic stress exposure in mice. Sleep disturbances are one of the most commonly reported symptoms by people with post-traumatic stress disorder (PTSD). The present study aims at investigating whether SSR125543 (10 mg/kg/day/i.p. for 2 weeks) is able to attenuate sleep/wakefulness impairment induced by traumatic stress exposure in a model of PTSD in mice using electroencephalographic (EEG) analysis. Effects of SSR125543 were compared to those of the 5-HT reuptake inhibitor, paroxetine (10 mg/kg/day/i.p.), and the partial N-methyl-D-aspartate (NMDA) receptor agonist, D-cycloserine (10 mg/kg/day/i.p.), two compounds which have demonstrated clinical efficacy against PTSD. Baseline EEG recording was performed in the home cage for 6 h prior to the application of two electric foot-shocks of 1.5 mA. Drugs were administered from day 1 post-stress to the day preceding the second EEG recording session, performed 14 days later. Results showed that at day 14 post-stress, shocked mice displayed sleep fragmentation as shown by an increase in the occurrence of both non-rapid eye movement (NREM) sleep and wakefulness bouts. The duration of wakefulness, NREM and REM sleep were not significantly affected. The stress-induced effects were prevented by repeated administration of SSR125543, paroxetine and D-cycloserine. These findings confirm further that the CRF<sub>1</sub> receptor antagonist SSR125543 is able to attenuate the deleterious effects of traumatic stress exposure.

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## 1. Introduction

Stress can have a significant negative impact on sleep and traumatic life events may produce at least temporary sleep disturbances that may include insomnia or subjective sleep problems [1]. There is

evidence which supports the idea that disrupted sleep represents a core component of post-traumatic stress disorder (PTSD), involved both in the development and the maintenance of this condition [2]. Sleep disturbances may exacerbate the difficulties associated with PTSD, especially those related to increase arousal such as concentration, hypervigilance or irritability. As an example, Meewise et al. [3] suggested that in victims of a major industrial fire, sleep disturbances may contribute to attentional dysfunction many years after the traumatic event. From these observations it was concluded that efficiently addressing post-traumatic sleep symptoms during PTSD

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may be beneficial in optimizing clinical interventions [4]. There have been relatively few studies to directly examine the impact of pharmacological intervention on sleep problems in PTSD [5]. Maher et al. [6] have reported an improvement of post-traumatic-induced sleep disturbances following selective serotonin reuptake inhibitors (SSRIs), which represent the mainstay of treatment of PTSD.

A potential new therapeutic approach to prevent post-traumatic sleep disturbances may be to reduce the activity of the corticotropin-releasing factor (CRF) system using CRF<sub>1</sub> receptor antagonists. Several studies have reported elevated levels of CRF in the cerebrospinal fluid [7,8] and in the plasma of PTSD patients [9]. CRF is well known to be the main physiological regulator of the stress response. Following exposure to emotional and/or physical stressors, it is synthesized in neurons of the paraventricular hypothalamic nucleus and triggers the secretion of adrenocorticotropin (ACTH), which subsequently stimulates the release of cortisol from the adrenal cortex into blood and exerts a negative feedback on the hypothalamic pituitary adrenal (HPA) axis [10,11]. Several studies indicate that CRF may play a role in the regulation of stress-induced changes in arousal and sleep. For example, Opp et al. [12] demonstrated that rat strains differing in the synthesis and secretion of CRF, and in basal plasma concentrations of corticosterone showed significant differences in the amounts of sleep. Specifically, Lewis rats, known to display a deficiency in the synthesis and secretion of hypothalamic CRF, exhibit less wakefulness and more NREM than the genetically-related inbred Fischer 344 and outbred Sprague–Dawley [12,13] and Wistar rats [13]. Other studies have reported that blockade of the CRF<sub>1</sub> receptor reduced stress-induced sleep disturbances. For instance, the infusion of the CRF<sub>1</sub> receptor antagonist, antalarmin, into the central nucleus of the amygdala resulted in an attenuation of electric shock-induced reduction in REM sleep in rats [14]. In another study using depressed subjects, four weeks of treatment with the CRF<sub>1</sub> receptor antagonist, R121919, decreased the number of awakenings [15]. Based on this observation, it can tentatively be suggested that a CRF<sub>1</sub> receptor antagonist would also be able to attenuate sleep disturbances in other stress-related conditions, such as PTSD.

We have recently demonstrated that the CRF<sub>1</sub> receptor antagonist, SSR125543, was able to attenuate several behavioral (cognitive deficit), hormonal (increase in corticosterone level) and electrophysiological (hippocampal excitability impairment) effects following exposure to a traumatic event [16,17]. However, potential beneficial effects of SSR125543 on stress-induced sleep disturbances have not been studied. Therefore, the aim of the present study was to investigate the effects of SSR125543 on long-term sleep disturbances in stressed mice. We used a model of PTSD [16] based on exposure of mice to unavoidable electric foot-shocks. Sleep/wakefulness was investigated by using electroencephalographic (EEG) recording, which was performed prior to shock application and 14 days after stress. Our previous findings have shown that the current procedure produced at day 14 post stress a fragmentation of sleep [18]. SSR125543 was administered repeatedly and its effects were compared to those of paroxetine and D-cycloserine (DCS), two clinically active compounds in PTSD patients.

## 2. Material and methods

### 2.1. Animals

Swiss male mice (Janvier, Le Genest St-Isle, France) weighing 20–22 g at the start of the experiment were used. They were housed individually in plastic cages (30 × 18 × 18 cm) with free access to food and water *ad libitum*. They were maintained at a constant

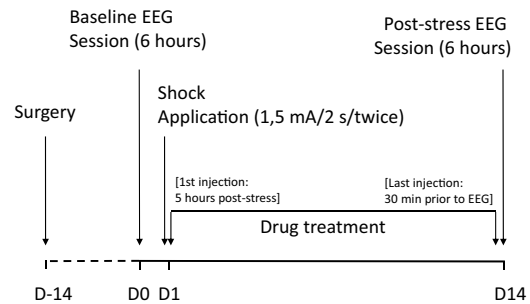


Fig. 1. Experimental design.

temperature of  $21 \pm 1^\circ\text{C}$ , humidity at  $50 \pm 10\%$  and under a 12:12 light/dark cycle (light on at 7:00 a.m.). Experiments were conducted in accordance with the “Guide and Care and Use of Laboratory Animals” (National Institute of Health) and were approved by the in-house Animal Ethics Committee.

### 2.2. Shock application

Animals were placed into the shock chamber for a 190-s habituation period after which two electric foot-shocks (1.5 mA; for 2 s; 6 s apart) were delivered through the metal grid floor. Animals remained in the shock chamber for another 60-s period before they were returned to their home cage. Control animals were exposed to the same procedure, but without receiving any foot-shock.

### 2.3. Drug administration

Paroxetine (Sigma-Aldrich, CAS 110429-35-1), D-cycloserine (Sigma-Aldrich, CAS 68-41-7), and SSR125543, synthesized by Sanofi Medicinal Chemistry, were suspended in saline with methylcellulose (0.6%) and Tween 80 (0.1%) to obtain concentrations of 1.0 mg/ml. The treatments began five hours after stress. Mice received one intraperitoneal (i.p.) administration per day of 10 ml/kg. The last administration was performed 30 min before the start of EEG recordings. The doses were validated in a previous study using the same procedure and the same species. It showed that 10 mg/kg represented the optimal dose to seek efficacy in this model [17].

### 2.4. Sleep/wakefulness analysis

#### 2.4.1. Surgery

Mice were anesthetized with Zoletil®50 (Tiletamine, Zolazepam, 60 mg/kg, i.p.), mounted in the stereotaxic apparatus and secured using blunt rodent ear bars. A scalp incision was made after local anesthesia with lidocaine 2% and the skin was retracted. The skull surface was cleaned to implant small stainless steel screw electrodes (0.9 mm in diameter). Three cortical electrodes were screwed into the bone over the sensorimotor cortex (1.5 mm lateral to the median and 1.5 mm behind the frontoparietal sutures), the visual cortex (1.5 mm lateral to the median and 1.5 mm in front of the parieto-occipital sutures) and over the cerebellum. They were attached to a connector (Winchester®, 5-led) and fixed with dental cement (3 M® ESPE) to the cranium. Animals were allowed to recover from surgery in their individual cage for two weeks prior recordings.

#### 2.4.2. Recording procedure

The experimental design is shown in Fig. 1. Mice were habituated in their home cage to the recording cable and room for one day prior to each EEG recording session. On the recording day, they were connected to the cable at 9:45 a.m. Recording sessions took

place in the home cage between 10:00 a.m. and 04:00 p.m. and lasted 6 h. Baseline EEG parameters were recorded before the stress procedure. A second EEG recording session was performed 14 days following shock application. Groups of 5–7 mice were used for each treatment. In addition, a group of non-stressed mice were used to control the stability of the EEG baseline over a 14-day period.

#### 2.4.3. Signal processing and sleep parameters

Implanted mice were connected to an EEG recording system (2 Grass, 12 tracks, 79D model) by a flexible cable with a rotating collector (APCL 12 channels, Air precision), which allowed mice to move freely. EEG signals were filtered at 1 and 100 Hz (6 dB/octave). They were then acquired and digitized at 256 Hz using the software Coherence 32 (Deltamed). Activities in the sensorimotor and visual cortices were recorded over the 6-h recording period by comparison with the reference electrode placed over the cerebellar cortex. Three sleep/wakefulness states were considered: (1) wakefulness was characterized by low voltage EEG signal and fast frequency (theta rhythm: within the 6–9 Hz range) on both cortical derivations; (2) non-rapid eye movements sleep (NREMS) were characterized by high voltage with slow wave (delta rhythm: within the 1–4 Hz range) with bursts of sleep spindles (sigma rhythm: within the 10–15 Hz range) on the sensorimotor derivation; (3) rapid eye movement sleep (REMS) by hypersynchronization of the theta rhythm (within the 4–9 Hz range) in the visual area. Analysis of the EEG signal was performed automatically by a computerized system discriminating between the various phases and visual control was also performed. The parameters examined were (1) total wakefulness time, (2) total NREMS time, (3) total REMS time, (4) mean duration of NREMS episodes, (5) mean number of NREMS episodes; (6) mean duration of wakefulness episodes, (7) mean number of wakefulness episodes, (8) mean duration of REMS episodes, and (9) mean number of REMS episodes over the 6-h recording sessions. Two-way ANOVA (treatment  $\times$  day) with repeated measures on factor day was used to assess overall effect of treatment, and Winer post test was used to compare differences between days.

### 3. Results

The details of the statistical analyses are summarized in Table 1. Results showed that the total durations of wakefulness, NREMS and REMS, as well as REMS episodes over the 6-h recording period on day 14 were not significantly affected by the application of unavoidable foot-shocks in any of the groups (Table 2 and Fig. 2A and B). However this stressor produced an increase in the number of wakefulness bouts in vehicle-treated mice (Fig. 3A). Pretreatment with paroxetine, DCS and SSR125543 prevented the increase in wakefulness bouts as post-stress performance in these groups were not significantly different from baseline levels. Similarly, the application of foot-shocks resulted in an increase in the occurrence of NREMS bouts (Fig. 4A). Again, this effect was prevented by the three drug treatments. While the occurrence of wakefulness and NREMS episodes was modified by stress, their average duration remained unchanged in all groups, regardless the condition (Figs. 3B and 4B). Finally, baseline performance in non-stressed vehicle-treated mice did not significantly differ between day 0 and day 14 for any of the recorded sleep/wakefulness parameters (Figs. 3 and 4).

### 4. Discussion

This study demonstrated that the selective non-peptide CRF<sub>1</sub> receptor antagonist, SSR125543, is able to prevent stress-induced increase of sleep fragmentation in a mouse model of PTSD. These effects were shared by the SSRI, paroxetine, and the NMDA receptor

**Table 1**  
Summary of statistical analyses using two-way ANOVA (DAY  $\times$  GROUP) with repeated measures on Factor DAY (Day 0 vs. Day 14).

Effect	Total wakefulness time	Total NREMS time	Total REMS time	Number wakefulness episodes	Mean duration wakefulness episodes	Number NREMS episodes	Mean duration NREMS episodes	Number REMS episodes	Mean duration REMS episodes
DAY	$F(1,24) = 3.3$ , ns	$F(1,24) = 3.8$ , ns	$F(1,20) = 3.2$ , ns	$F(1,24) = 3.9$ , ns	$F(1,24) = 1.3$ , ns	$F(1,24) = 0.7$ , ns	$F(1,24) = 0.5$ , ns	$F(1,20) = 5.6$ , $P = 0.03$	$F(1,20) = 1.5$ , ns
GROUP	$F(4,24) = 0.4$ , ns	$F(4,24) = 0.7$ , ns	$F(4,20) = 0.8$ , ns	$F(4,24) = 0.8$ , ns	$F(4,24) = 0.6$ , ns	$F(4,24) = 1.1$ , ns	$F(4,24) = 1$ , ns	$F(4,20) = 0.6$ , ns	$F(4,20) = 0.1$ , ns
DAY $\times$ GROUP	$F(4,24) = 1$ , ns	$F(4,24) = 0.6$ , ns	$F(4,20) = 0.5$ , ns	$F(4,24) = 3.5$ , $P = 0.03$	$F(4,24) = 0.8$ , ns	$F(4,24) = 2.7$ , $P = 0.05$	$F(4,24) = 0.8$ , ns	$F(4,20) = 0.4$ , ns	$F(4,20) = 0.8$ , ns

ns = P value is non-significant.

**Table 2**

Wakefulness, NREMS and REMS total duration prior (Baseline) and 14 days after the application of inescapable electric foot-shocks (Post-stress).

Condition	Treatment	Total wakefulness time (min)		Total NREMS time (min)		Total REMS time (min)	
		Baseline	Post-stress (D14)	Baseline	Post-stress (D14)	Baseline	Post-stress (D14)
Non-stressed	Vehicle	143.5 ± 15.1	146.0 ± 12.2	205.7 ± 14.1	204.5 ± 11.9	13.3 ± 1.7	8.8 ± 2.3
Stressed	Vehicle	128.2 ± 10.5	156.4 ± 13.6	222.2 ± 14.5	194.5 ± 14.3	13.1 ± 1.8	11.2 ± 1.9
	PAX	121.8 ± 15.9	149.8 ± 8.3	230.6 ± 14.9	203.7 ± 13.7	9.2 ± 1.7	9.6 ± 2.7
	DCS	138.8 ± 10.0	135.1 ± 9.7	208.8 ± 2.3	212.9 ± 9.4	11.3 ± 2.1	10.1 ± 1.7
	SSR	146.2 ± 12.9	151.9 ± 21.2	197.9 ± 11.3	197.3 ± 7.3	15.9 ± 2.4	11.5 ± 2.3

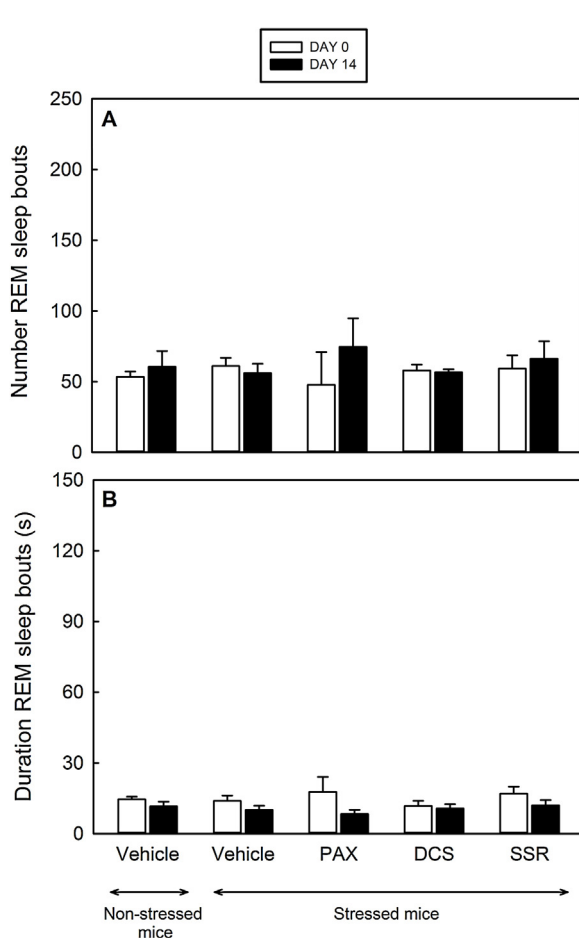
Paroxetine (PAX, 10 mg/kg i.p.), D-cycloserine (DCS, 10 mg/kg i.p.) and SSR125543 (SSR, 10 mg/kg i.p.) were administered once-a-day for 14 days after electric shock application. Data were recorded during light period before stress and 14 days after stress exposure and are expressed as total wakefulness, total NREMS and total REMS means (±S.E.M.).

partial agonist, DCS, two compounds with proven efficacy in the clinical management of PTSD.

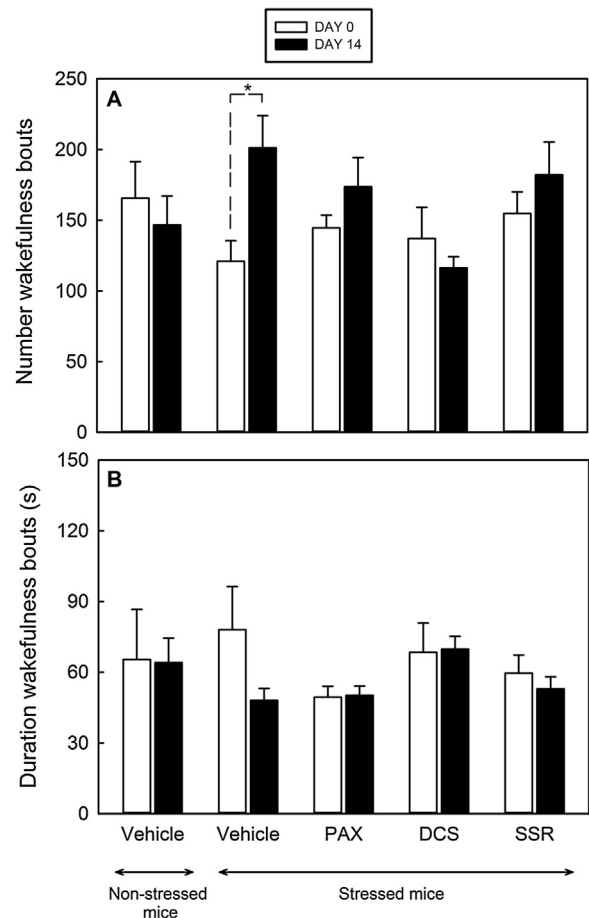
Consistent with our previous findings [18], exposing mice to two unavoidable foot-shocks of 1.5 mA intensity enhanced sleep fragmentation during the light period, as shown by the increase of NREMS and wakefulness episodes 14 days after the stress procedure. Further analysis of NREMS and wakefulness showed that stress had no significant effect on the total amount of any of these states. This finding parallels human studies reporting that sleep fragmentation reduces the quality of NREM sleep in PTSD patients without interfering with total NREM sleep time [1]. However it is important to emphasize that unlike to what is generally observed in human [2] and in animal procedures of fear conditioning in rats, REMS was not affected by stress in the current study, suggesting

only partial face validity of our procedure as a model of sleep alterations in PTSD. The reason for this discrepancy is unclear but it is possible that REMS of mice – the species used here – may be less sensitive to the effects of stress.

Sleep fragmentation interferes with the architecture of normal sleep and impairs the restorative/cognitive benefits of sleep [19]. It leads to cognitive impairments even though total sleep time may not be affected [20,21]. It was suggested that loss of NMDA receptor-dependent long-term potentiation in the hippocampal CA1 region may be one mechanism involved in this deficit [22]. This hypothesis is compatible with the findings from our previous study using the same stress procedure as employed here, which showed a deficit in episodic memory associated with a decrease of

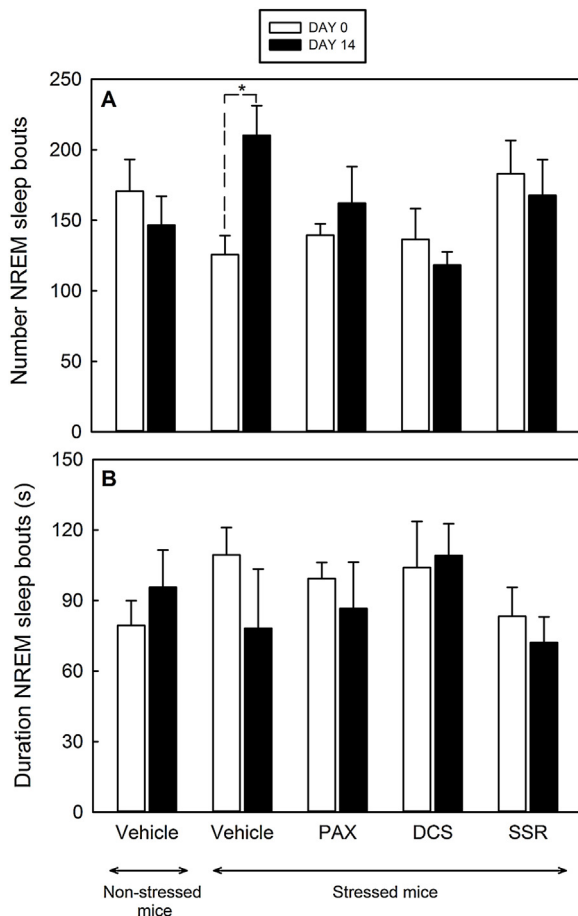


**Fig. 2.** Effects of 2-week treatment of paroxetine (PAX, 10 mg/kg, i.p.), D-cycloserine (DCS, 10 mg/kg, i.p.) and SSR125543 (SSR, 10 mg/kg, i.p.) on the long-term consequences on REMS bouts of two unavoidable electric shocks applied 14 days before. Each bar represents the average (±S.E.M.) (A) occurrence or (B) duration of REMS bouts during the 6-h EEG recording session.  $N = 5$  to 7 animals per group.



**Fig. 3.** Effects of 2-week treatment of paroxetine (PAX, 10 mg/kg, i.p.), D-cycloserine (DCS, 10 mg/kg, i.p.) and SSR125543 (SSR, 10 mg/kg, i.p.) on the long-term consequences on wakefulness bouts of two unavoidable electric shocks applied 14 days before. Each bar represents the average (±S.E.M.) (A) occurrence or (B) duration of wakefulness bouts during the 6-h EEG recording session. \* $P < 0.05$ , Day 14 significantly different from Day 0.  $N = 5$  to 7 animals per group.





**Fig. 4.** Effects of 2-week treatment of paroxetine (PAX, 10 mg/kg, i.p.), D-cycloserine (DCS, 10 mg/kg, i.p.) and SSR125543 (SSR, 10 mg/kg, i.p.) on the long-term consequences on NREMS bouts of two unavoidable electric shocks applied 14 days before. Each bar represents the average ( $\pm$  S.E.M.) (A) occurrence or (B) duration of NREMS bouts during the 6-h EEG recording session. \*  $P < 0.05$ , Day 14 significantly different from Day 0.  $N = 5$  to 7 animals per group.

hippocampal CA1 excitability 14 days post-stress [17]. It is likely, based on our current results, that these cognitive-related events following stress may have been at least in part due to an increase in sleep fragmentation.

The pharmacological experiments demonstrated that sleep fragmentation following stress can be prevented by repeated treatment with paroxetine. To the best of our knowledge, this is the first study which reported preventing effects of an SSRI on long-lasting sleep disturbances following acute stress in mice. SSRIs, including paroxetine, are commonly used to treat PTSD, and evidence suggests that they have a small but significant positive effect on sleep disruption (for reviews, see [5,23]). For example, in a placebo-controlled trials of 12 weeks duration which evaluated the efficacy of paroxetine for PTSD, the drug was reported to reduce the severity of distressing dreams and the difficulty falling or staying asleep in PTSD patients [24]. However, effects of paroxetine or other SSRIs on sleep fragmentation in PTSD patients have not been reported yet.

The selective partial agonist of the NMDAR, DCS, prevented the long-term effects of stress on sleep/wakefulness by normalization sleep fragmentation. Clinical studies examining the effects of DCS have reported that this compound both promoted the extinction of fear and protected against reinstatement of fear in patients suffering from PTSD [25], and facilitated fear extinction in other anxiety disorders such as acrophobia [26] or social anxiety disorder [27]. Moreover, several studies reported a greater symptom reduction in

PTSD patients receiving DCS compared to those from the placebo group during exposure therapy [28–31]. These clinical findings are substantiated by experiments in rodents, which showed that DCS promotes fear extinction [32–34]. It is noteworthy that contextual fear extinction was shown to ameliorate fear conditioning-induced sleep disturbances in rats [35]. The authors of this study concluded that sleep disturbances in PTSD may be improved by therapies that address and eliminate the associated fear, an idea in line with the current findings with DCS on stress-induced sleep fragmentation. The mechanisms involved in the positive effects of DCS on sleep architecture modifications after unavoidable stress and more generally on behavioral alterations following such events remain to be defined precisely. Gupta and colleagues [36] have demonstrated that the efficacy of DCS on fear extinction may be partly due to its ability to affect neuronal activity and signalling in the medial prefrontal cortex and amygdala subnuclei as shown by the increase in phospho-ERK, a marker for synaptic plasticity, and the increase in expression of ionotropic glutamate receptors. Moreover, the action of DCS on trauma-related responses may involve other brain regions, including the hippocampus. Several studies have reported that DCS treatment normalises stress-impaired synaptic transmission in the CA1 field of the hippocampus, via NMDAR activation leading to increased synaptic plasticity, which resulted in a restoration of memory performance [17,32–34]. It is possible that some of these mechanisms may have been involved in the current effects of DCS on stress-induced sleep fragmentation.

Similar to paroxetine and DCS, the CRF<sub>1</sub> receptor antagonist, SSR125543, was able to prevent the protracted effects of unavoidable stress on sleep fragmentation. The literature describing effects of CRF<sub>1</sub> receptor antagonists on stress-induced sleep/wakefulness alterations is sparse. As indicated above, Liu et al. [14] showed that microinjection of antalarmin into the central nucleus of the amygdala attenuated fear-induced reductions in REMS in a fear-conditioning paradigm in rats. The current observation that REMS was not affected in our stress procedure in mice does not enable a direct comparison between both studies, but it emphasizes that central blockade of CRF<sub>1</sub> receptors may alleviate the deleterious effects of stress on sleep. Other findings reinforce the idea that the CRF system plays an important role in the regulation of sleep/wakefulness under stressful conditions. For example, Romanowski et al. [37] reported that intracerebroventricular infusion of the CRF peptide, which is well-known to produce anxiogenic effects in animals and in humans, produced a decrease in the length of NREMS episodes in mice, an effect not seen in mice lacking the central CRF<sub>1</sub> receptor. SSR125543 has been demonstrated to produce its effects on stress-induced responses via an action at both hypothalamic and central CRF<sub>1</sub> receptors [16,38]. For example, the involvement of the former was evidenced by the ability of the drug to prevent restraint stress-induced elevation of ACTH levels in rats [38], while the implication of central CRF<sub>1</sub> receptors was demonstrated by the ability of SSR125543 to attenuate the cognitive effects of stress in mice whose HPA axis was blunted by pharmacological means [16]. Whether the current effects of SSR125543 involve an action at hypothalamic or central CRF<sub>1</sub> receptors, or both, remains to be established. The aforementioned findings [14,37] that central modulation of the CRF system is able to modify sleep/wakefulness tend to support an involvement of central CRF<sub>1</sub> receptors in this regulation. This idea is further strengthened by the observation that conditional CRF overexpression in the mouse forebrain enhances REMS in the absence of HPA axis alteration [39].

The pharmacological treatments in the current study were applied chronically to mimic more closely the clinical situation in PTSD where drugs are generally administered repeatedly to achieve significant efficacy. However, since in this study the last administration of each drug was performed 30 min before testing for sleep, it

cannot be totally excluded that a single injection of the drugs may have been sufficient to reach a similar effect. It would be worth testing this idea in future studies.

In conclusion, the findings of the present study strengthen further the idea that the long term effects of brief unavoidable electric shocks may have relevance for certain aspects of human PTSD. Furthermore, they suggest that the NMDA receptor partial agonist, DCS, in addition to its promoting effects on fear extinction, may also have the potential to alleviate the sleep symptoms in PTSD. Finally, these findings support further the therapeutic potential of CRF<sub>1</sub> receptor antagonists in treating PTSD patients.

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